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Indian Standard
SPECIFICATION FOR
WARFARIN, TECHNICAL

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Indian Standard

SPECIFICATION FOR WARFARIN, TECHNICAL

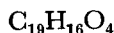
0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 27 February 1970, after the draft finalized by the Pest Control Sectional Committee had been approved by the Agricultural and Food Products Division Council and the Chemical Division Council.

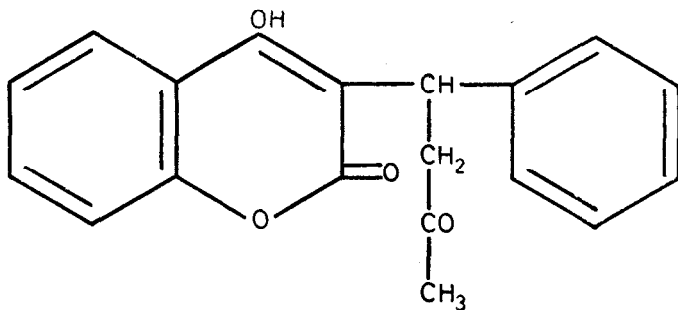
0.2 Warfarin, technical, is used in the preparation of formulations for the control of rodent pests, such as rats, mice and bandicoots. The material acts as an hemorrhagic agent or as an anticoagulant, which, when eaten for a period of time, causes death due to hemorrhage in the blood system.

0.2.1 Warfarin is the accepted common name by the International Organization for Standardization, for the pesticide containing 3-acetonyl benzyl-4-hydroxycoumarin as its active ingredient. The structural and chemical formulae and the molecular weight of this compound are indicated below:

Empirical Formula



Structural Formula



Molecular Weight

308.0

0.3 Warfarin, technical, is known to have two impurities, namely, Alice's ketone [3-(O-hydroxyphenyl)-5-phenyl-1-2 cyclohexene-1-one] and benzalacetone and alkali insolubles. These phenolic ketones, when present beyond a certain limit, tend to repel the rats from accepting the bait

containing this product. The limits for these impurities have been established in some overseas standards and some investigations have also been carried out in the country by feeding Indian rats with warfarin containing variable quantities of Alice's ketone. On the basis of the results of the existing investigations, this standard prescribes the maximum limit of Alice's ketone as 750 ppm in warfarin, technical. This limit may have to be amended after the investigations that are still in progress have been completed. When these investigations are completed, a limit for benzalacetone and alkali insolubles may also require to be specified.

0.4 Taking into consideration the views of producers, consumers, testing authorities and technologists, the Sectional Committee responsible for the preparation of this standard felt that it should be related to the manufacturing and trade practices followed in the country in this field.

0.5 This standard is one of a series of Indian Standards on pesticides and their formulations.

0.6 This standard contains clauses **3.1**, **D-2.3** and **D-3.4** which call for an agreement between the purchaser and the vendor.

0.7 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS:2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard prescribes the requirements and the methods of test for warfarin, technical, used in making rodenticide formulations.

2. REQUIREMENTS

2.1 The material shall be in the form of free-flowing, white or light tan, crystalline powder, free from any lumps, extraneous impurities, added modifying agents or odours.

2.2 The material shall comply with the requirements specified in Table 1.

3. PACKING AND MARKING

3.1 The material shall be packed in clean and dry air-tight containers made of galvanized steel sheet, tinplate, steel, glass or plastic as agreed to between the purchaser and the manufacturer.

*Rules for rounding off numerical values (revised).

TABLE 1 REQUIREMENTS FOR WARFARIN, TECHNICAL

(Clause 2.2)

Sl No.	CHARACTERISTIC	REQUIREMENT	METHODS OF TEST, REF TO APPENDIX
(1)	(2)	(3)	(4)
i)	3-acetonylbenzyl-4-hydroxycoumarin content, percent by weight, <i>Min</i>	98.0	A
ii)	Melting point, °C (<i>see</i> Note)	159 to 162	B
iii)	Alice's ketone, ppm, <i>Max</i>	750	C

NOTE — The first sign of melting of the material taken for the test could be at any temperature within the range as given under col 3. However, the difference between the temperatures at which the first sign of melting and the completion of melting of the whole material that is taken for the test, shall not exceed 3°C.

3.2 The containers shall be securely closed and sealed air-tight after filling them with the material and shall bear legibly and indelibly the following information:

- a) Common name of the material;
- b) Name of the manufacturer;
- c) Date of manufacture;
- d) Batch number;
- e) Net weight of the contents;
- f) Active ingredient content, percent by weight;
- g) The minimum cautionary notice worded as under:

‘DANGEROUSLY HAZARDOUS. KEEP THE MATERIAL AND BAITS CONTAINING THE MATERIAL AWAY FROM CHILDREN, DOMESTIC ANIMALS, FOODSTUFFS, ANIMAL FEEDS. DO NOT USE THE EMPTY CONTAINER FOR STORAGE OF FOODSTUFFS OR FEEDS.

ANTIDOTE: MASSIVE DOSES OF VITAMIN K AND IF FOUND NECESSARY BLOOD MAY BE TRANSFUSED.’

3.2.1 In addition to the above, the container shall be marked with the symbol for danger of poisoning as specified in IS:1260-1958* and the word ‘POISON’ in distinct, bold capital letters shall be printed.

*Code of symbols for labelling of dangerous goods.

3.2.1.1 The container may also be marked with the ISI Certification Mark.

NOTE — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act, and the Rules and Regulations made thereunder. Presence of this mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard, under a well-defined system of inspection, testing and quality control during production. This system, which is devised and supervised by ISI and operated by the producer, has the further safeguard that the products as actually marketed are continuously checked by ISI for conformity to the standard. Details of conditions, under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

4. SAMPLING

4.1 Representative samples of the material shall be drawn as prescribed in Appendix D.

5. TESTS

5.1 Tests shall be carried out as prescribed in the appropriate appendices as specified in col 4 of Table 1.

5.2 Quality of Reagents — Unless specified otherwise, pure chemicals and distilled water (*see* IS: 1070-1960*), shall be employed in the test.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

A P P E N D I X A

[*Table 1, Item (i)*]

DETERMINATION OF 3-ACETONYLBENZYL- 4-HYDROXYCOUMARIN

A-1. APPARATUS

A-1.1 Spectrophotometer — Model DU, Beckman or equivalent instrument with 1-cm quartz cells.

A-2. REAGENT

A-2.1 Standard Sodium Hydroxide Solution — 0.1 N.

*Specification for water, distilled quality (*revised*).

A-3. PROCEDURE

A-3.1 Weigh accurately about 125 mg of the sample into a 250-ml volumetric flask, add standard sodium hydroxide to affect solution and make up to the mark with the same. Dilute 2 ml of this solution to 100 ml with standard sodium hydroxide solution. With the spectrophotometer set at maximum sensitivity, determine the absorption of the final solution at 308 m μ using standard sodium hydroxide as reference.

A-4. CALCULATION

$$\begin{aligned} \text{A-4.1 } 3\text{-acetonylbenzyl-4-hydroxycoumarin} \\ \text{content, percent by weight} &= \frac{E \times 308.3 \times 100 \times 100 \times 100}{1.42 \times 10\,000 \times 1\,000 \times 2 \times W} \\ &= \frac{E \times 10.86 \times 2.5}{W} \end{aligned}$$

where

E = absorption of the final solution at 308 m μ , and

W = weight of sample (g).

NOTE 1 — Molecular weight of warfarin, technical, is 308.3. Molar extinction coefficient = 1.42×10^4 .

NOTE 2 — The molar extinction coefficient shall be determined with power 3-(α -phenyl- β -acetyethyl)-4-hydroxycoumarin, the value given here is an example only.

APPENDIX B

[Table 1, Item (ii)]

DETERMINATION OF MELTING POINT BY CAPILLARY TUBE METHOD

B-1. EQUIPMENT

B-1.1 Thermometer — a long-stem short-bulb thermometer with a range of 0° to 200°C (or better 100° to 200°C) and divisions of 0.5°C.

B-1.2 Bath and Heating Assembly — This consists of a suitable housing with a glass front. Inside, there is a long-necked flask (250-ml Kjeldahl flask) three-fourths full with liquid paraffin and placed on a suitable mounting for heating with a gas burner. The flask is loosely fitted with a stopper which carries a thermometer. The housing is provided with appropriate illumination for the observation of melting and reading of temperature.

B-2. PROCEDURE

B-2.1 Take a small quantity of the finely powdered prepared sample in the capillary tube with one end sealed. Ensure proper packing of the material in the tube by gently tapping on the table. The material in the capillary tube should be *sufficient to cover the entire length of the thermometer bulb*. Heat the bath with a small flame comparatively rapidly to about 125°C. Attach the capillary tube with the thermometer such that the material is very close to the bulb. Introduce the thermometer in the bath in such a way that the thermometer bulb is well below the surface of the liquid. Now heat slowly and carefully so that the rise in bath temperature is about 1°C per minute. Note the temperatures of initial melting (when first liquid drop appears) and completion of melting (when no solid particle is left). Report the temperature of melting point completion and the difference of two temperatures as melting point range.

NOTE — Ensure that two consecutive determinations do not differ by more than 0.5°C.

APPENDIX C

[Table 1, Item (iii)]

DETERMINATION OF ALICE'S KETONE

C-1. PROCEDURE

C-1.1 Dissolve 1.17 g of sample of warfarin in 10 ml of 5 percent aqueous sodium hydroxide solution. Determine the optical density in a Beckman DU spectrophotometer (or similar instrument) at 385 m μ through a 1-cm light path. The ppm of 'Alice's ketone' is 380 \times optical density.

APPENDIX D

(Clause 4.1)

SAMPLING OF WARFARIN, TECHNICAL

D-1. GENERAL REQUIREMENTS

D-1.0 In drawing, preparing, storing and handling test samples, the following precautions and directions shall be observed.

D-1.1 Samples shall not be taken in an exposed place.

D-1.2 The sampling instrument shall be clean and dry when used.

D-1.3 Proper precautions shall be taken while drawing samples since the material is toxic.

D-1.4 Precautions shall be taken to protect the samples, the material being sampled, the sampling instruments and the receptacles for samples from adventitious contamination.

D-1.5 To draw a representative sample, the contents of each container selected for sampling shall be mixed as thoroughly as possible by shaking or by any other suitable means so as to bring all portions into uniform distribution.

D-1.6 The samples shall be placed in suitable, clean, dry and air-tight sample receptacles.

D-1.7 The sample receptacles shall be of such a size that they are almost, but not completely, filled by the sample.

D-1.8 Each sample receptacle shall be sealed air-tight after filling and marked with full details of sampling, the date of manufacture, name of the manufacturer and other particulars of the consignment.

D-1.9 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from the normal temperature.

D-2. SCALE OF SAMPLING

D-2.1 Lot—All the containers in a single consignment of the material drawn from the same batch of manufacture shall constitute a lot. If a consignment is declared or is known to consist of different batches of manufacture, the containers belonging to the same batch shall be grouped together and each such group shall constitute a separate lot.

D-2.1.1 Samples shall be tested for each lot for ascertaining the conformity of the material to the requirements of the specification.

D-2.2 The number (n) of containers to be chosen from the lot shall depend on the size of the lot and shall be in accordance with col 1 and 2 of Table 2.

D-2.3 These containers shall be chosen at random from the lot and in order to ensure the randomness of selection, some random number table as agreed to between the purchaser and the vendor shall be used. In case such a table is not available, the following procedure shall be adopted:

Starting from any container in the lot, count them as 1, 2, 3, etc, up to r in a systematic manner, where r is equal to the integral part

of the value of N/n , N being the total number of containers in the lot and n the number of containers to be chosen (*see* Table 2). Every r th container thus counted shall be separated until the requisite number of containers is obtained from the lot to give samples for test.

TABLE 2 NUMBER OF CONTAINERS TO BE CHOSEN FOR SAMPLING

(Clauses D-2.2 and D-2.3)

LOT SIZE	NO. OF CONTAINERS TO BE CHOSEN
N	n
(1)	(2)
3 to 15	3
16 „ 40	4
41 „ 65	5
66 „ 110	7
Over 110	10

D-3. TEST SAMPLES AND REFEREE SAMPLES

D-3.1 Before drawing the test sample, thoroughly mix the contents of each container selected by shaking or by any other suitable means. Draw small portions of the material from different parts of each container selected (*see* Table 2). The total quantity of the material drawn from each container shall be sufficient to conduct the tests for all the characteristics given in Table 1 and shall be not less than 400 g.

D-3.2 Mix thoroughly all portions of the material drawn from the same container. Out of these portions, a small but equal quantity shall be taken for each selected container and shall be well mixed together so as to form a composite sample of not less than 750 g. This composite sample shall be divided into three equal parts, one for the purchaser, another for the vendor and the third for the referee.

D-3.3 The remaining portions of the material from each container (after a small quantity needed for formation of the composite sample has been taken out) shall be divided into three equal parts. These parts shall be immediately transferred to thoroughly dried sample receptacles which are then sealed air-tight, and labelled with all the particulars of sampling given under **D-1.8**. The material in each such sealed sample receptacle shall constitute a test sample. These individual samples shall be separated into three identical sets of test samples in such a way that each set has a sample representing each container selected (*see* Table 2). One of these three sets shall be marked for the purchaser, another for the vendor and the third for the referee.

D-3.4 Referee samples shall consist of the composite sample (*see* **D-3.2**) and a set of individual test samples (*see* **D-3.3**) marked for this purpose and shall bear the seals of the purchaser and the vendor. These shall be kept at a place agreed to between the two.

D-4. NUMBER OF TESTS

D-4.1 Tests for the determination of 3-acetyl benzyl-4 hydroxycoumarin content and Alice's ketone shall be conducted individually on each of the samples constituting a set of test samples (*see* **D-3.3**).

D-4.2 Tests for the determination of the remaining characteristic, namely, melting point, shall be conducted on the composite sample as prepared under **D-3.2**.

D-5. CRITERIA FOR CONFORMITY

D-5.1 A lot shall be declared as conforming to the specification if **D-5.1.1** and **D-5.1.2** are satisfied.

D-5.1.1 The values of the test results on the composite sample for melting point shall satisfy the corresponding requirement given in Table 1.

D-5.1.2 The values of the test results for 3-acetyl benzyl-4 hydroxycoumarin content and Alice's ketone, shall be recorded as shown in Table 3. The mean and range for the test results are calculated as below:

$$\text{Mean } (\bar{X}) = \frac{\text{Sum of the values of the test results}}{\text{Total number of test results}}$$

$$\text{Range } (R) = \text{Difference between the highest and the lowest value obtained for the test results.}$$

The appropriate expression as shown in col 6 of Table 3 shall be calculated. If the values of the expression satisfies the relevant condition as given in col 6 of Table 3, the lot shall be declared to have satisfied the requirement for 3-acetylbenzyl-4 hydroxycoumarin content and Alice's ketone.

TABLE 3 CRITERION FOR CONFORMITY

SL No.	CHARACTERISTICS	TEST RESULTS	MEAN	RANGE	CRITERION FOR CONFORMITY
(1)	(2)	(3)	(4)	(5)	(6)
i)	3-acetylbenzyl-4-hydroxycoumarin content	—	\bar{X}	R	$(\bar{X} - 0.6R) \geq 98$
ii)	Alice's ketone	—	\bar{X}	R	$(\bar{X} + 0.6R) \leq 750$

(Continued from page 2)

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